Revista Brasileira de Entomologia 59 (2015) 240–250

REVISTA BRASILEIRA DE
Entomologia
A Journal on Insect Diversity and Evolution
www.rbentomologia.com

Medical and Veterinary Entomology

Morpho-histological characterization of immature of the bioindicator midge *Chironomus sancticaroli* Strixino and Strixino (Diptera, Chironomidae)

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ARTICLE INFO

Article history:
Received 12 February 2015
Accepted 18 April 2015
Available online 18 July 2015
Associate Editor: Gustavo Graciolli

Keywords:
Ecotoxicology
Histological biomarker
Sediment

ABSTRACT

Chironomidae immature are used as bioindicators of sediment quality in aquatic ecosystems and ecotoxicological assays. Histological descriptions for this family are outdated and limited and there are no studies with Neotropical species. The aim of this study was to describe the tissue architecture of several organs of the larva of *Chironomus sancticaroli*. For the description of the histological pattern, the larvae were fixed in Duboscq solution for insects at 56 °C, followed by routine histologic processing, infiltration in paraffin, and the sections were stained with Hematoxylin–Eosin. After examining the slides, the tube digestive, salivary gland, excretory, nervous, endocrine, circulatory, and integumentary systems and fat body were histologically characterized. The histology allows evaluation of cell morphology, and for being not expensive and easily accessible can be routinely used in biomonitoring. In addition, is a useful tool in ecotoxicological assays and allow to evaluate biomarkers at tissue and cell levels.

Introduction

The immature forms of Chironomidae are abundant and widely distributed in water bodies, having their habits closely related to the sediment they are inserted in. One of their characteristics is the presence of hemoglobin in the hemolymph, which allows these organisms to tolerate low oxygen concentrations in the water (Trivinho-Strixino, 2011a). Another aspect is the possibility that this pigment is able to metabolize xenobiotics (Weber and Vinogradov, 2001; Ha and Choi, 2008), making possible the presence of these insects for a longer time in degraded environments. Thus, sublethal effects can be assessed by biomarkers of environmental contamination. As a result of these characteristics, these organisms are considered biondicators of environmental quality.

Several studies that applied biochemical and molecular biomarkers were performed using chironomids (Callaghan et al., 2001; Callaghan et al., 2002; Crane et al., 2002; Lee and Choi, 2006; Printes et al., 2007). However, there is a lack of application of cell and tissue biomarkers that demonstrate chronic effects.

Microscopic analyses can identify alterations in cells and tissues, and help in understanding the physiology of organs and systems. The microscopic technique is widely used to study fish in aquatic ecotoxicology (Bernet et al., 1999), being sensitive to detect subtle effects of pollutants, determination of target organs and possible toxic mechanisms. Because there are no sufficiently detailed histological descriptions of the organs and systems of chironomids and other species also used in toxicity tests, the histological description of representatives of this taxon becomes relevant.

Chironomus sancticaroli Strixino and Strixino, 1981 was considered synonymy to *Chironomus xanthus*, Rempel, 1939 (Trivinho-Strixino, 2011b), being easily reared in the laboratory (Fonseca and Rocha, 2004). They were used in ecotoxicological tests for the evaluation of environmental quality in Brazil (Moreira-Santos et al., 2005; Silvério et al., 2005; Dornfeld et al., 2006; Printes et al., 2007; Santos et al., 2007; Sotero-Santos et al., 2007; Janke et al., 2011; Printes et al., 2011; Novelli et al., 2012). To properly understand the effects of xenobiotics on different reduced scale body tissues such as Chironomidae larvae, there is need for prior knowledge of anatomical standard of the tissue before indication of any change. This study aims to describe the structure of the

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http://dx.doi.org/10.1016/j.rbe.2015.07.003

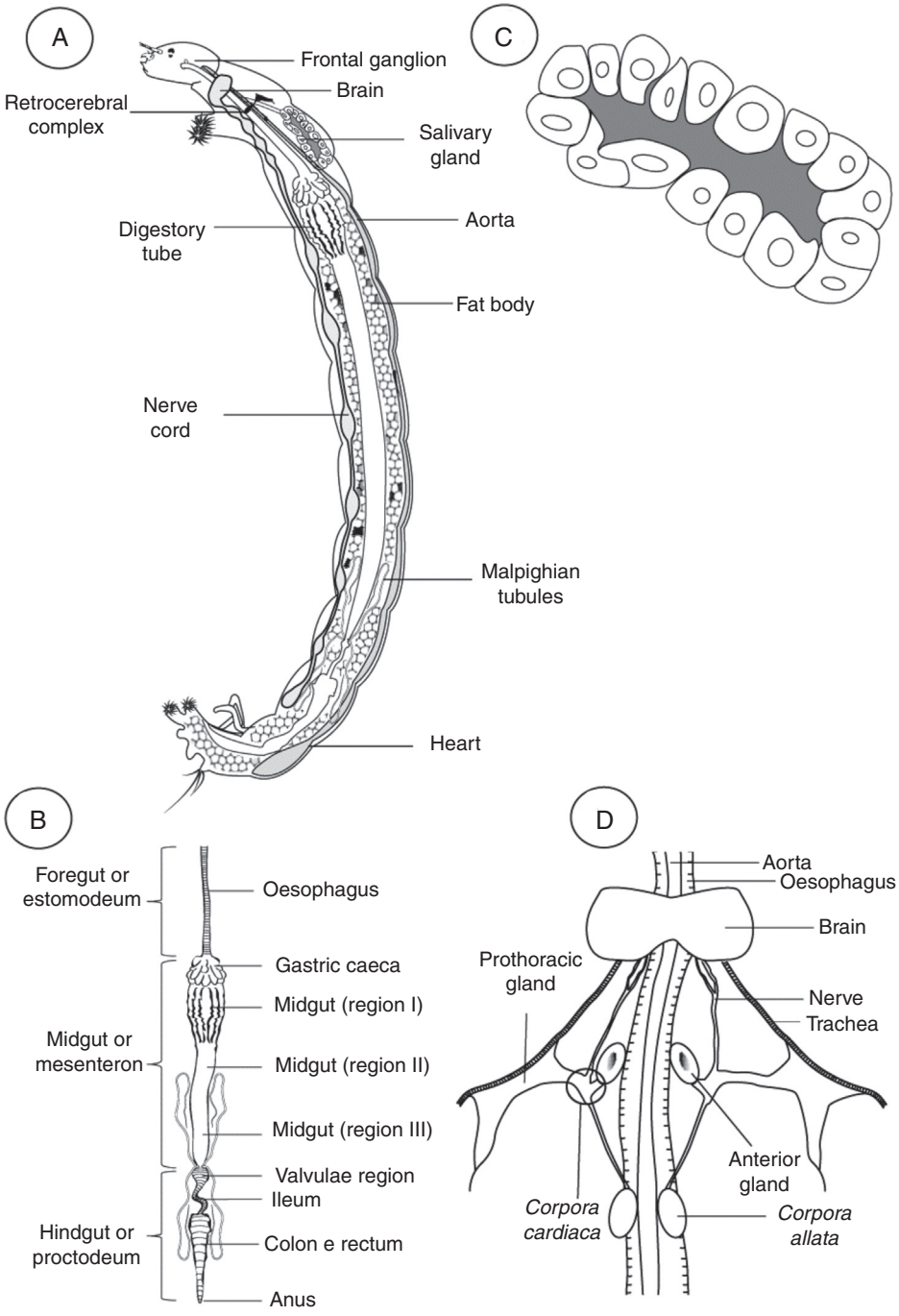

Fig. 1. Schematic representations of organs and tissues of immature *Chironomus sancticaroli*. (A) Internal morphology of the larva; (B) morphology of the digestive tract; (C) aspect of the salivary gland; (D) disposition of the endocrine glands in the retrocerebral complex.

standard tissue of different organs and systems of the larvae of *C. sancticaroli* and to offer subsidies for the use of microscopic techniques in these organisms for environmental analysis.

Material and methods

Larvae of *C. sancticaroli* were from a colony maintained at a breeding room of the Department of Zoology at the Universidade Federal do Paraná under controlled conditions of temperature ($25 \pm 2\,^{\circ}C$), humidity ($80 \pm 10\%$) and photoperiod (12 h light/12 h dark), constant aeration and TetraMin® crushed food, at the rate of 0.1 g per liter of dechlorinated water. We used 30 initial fourth instar larvae for histological description of the larval stage. The breeding of the species was adapted from Maier et al. (1990). The specimens were deposited in the Padre Jesus Santiago Moure Entomological Collection at the Universidade Federal do Paraná (DZUP), numbers 249269–249276.

Larvae were fixed in Duboscq solution for insects (Barth, 1958), for 4 h in an incubator at 56 °C. After fixation, the larvae were washed in 70% ethanol for the removal of the fixative solution and then the procedures of routine histological techniques were followed: the material was dehydrated in alcohol series, diaphanization in xylene and infiltration in paraffin.

We obtained transversal, lateral, longitudinal and dorsoventral sections of the larvae, in the thickness of 7 μm, and stained with Hematoxylin–Eosin. To perform the analysis of the material, all

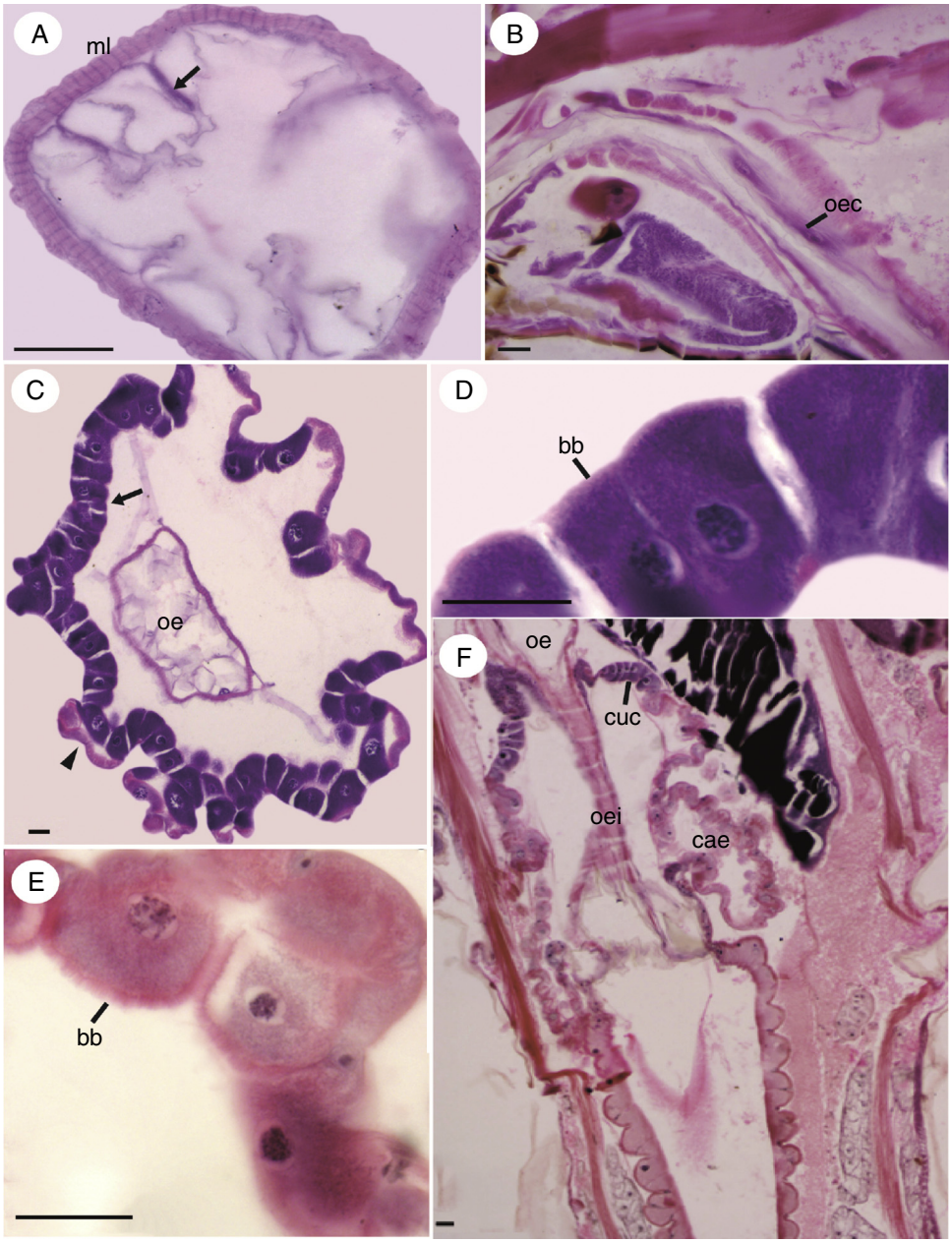

Fig. 2. Micrographs of the foregut and gastric caeca region of immature *Chironomus sancticaroli*. (A) Cross-section of the esophagus with longitudinal folds formed by the epithelium (arrow) and the longitudinal muscle layer; (B) longitudinal section showing the oesophageal lining epithelium (arrow); (C) cross-sectional of the caecum of the larva, note the Cuénot cells (arrow), cells of the caecum (arrowhead) and esophagus; (D) the Cuénot cells are detailed; (E) in detail, cells of gastric caeca; (F) longitudinal section the region of the caecum showing the estomodeal valve. bb: brush border; cae: gastric caeca; cuc: Cuénot cells; ml: muscle layer; oe: esophagus; oec: esophagus cells; oei: esophagus invagination. Stain: Harris hematoxylin and eosin. Scale bar = 20 μm.

histological sections were used to allow a better description of the standard tissue of different organs and systems.

Results

As initial parameter, we elaborated the description of the tissue pattern of organs and structures of the digestive system, salivary gland, excretory, nervous and endocrine system or retrocerebral complex, fat body and the circulatory and integumentary systems of larvae of *C. sancticaroli* (Fig. 1A).

Digestive system

The digestive tract of the *C. sancticaroli* larvae extends from mouth to anus, being divided into three regions: foregut, midgut and hindgut (Fig. 1B). The esophagus is the first region of the digestive tract, which corresponds to the region of the foregut. It is a short organ that shows no differentiated region, as crop or gizzard. This organ extends from the mouth to the metathorax. In cross-section, it is possible to observe five longitudinal folds of the epithelium, in addition to the thick muscular layer (Fig. 2A). In its initial portion, it is surrounded by a muscular structure known as periesophageal collar (Fig. 2B). The epithelium of difficult observation consists in pavement cells that do not exceed 6 mm thick, with basophilic cytoplasm and large nucleus (Fig. 2B).

Cuénot cells that are associated with the oesophageal projection are grouped and exhibit a reduced size compared to cells that are not associated to the projection. The cells have large spherical nuclei, strongly basophilic and granular cytoplasm and cell apex with brush border (Fig. 2C and D).

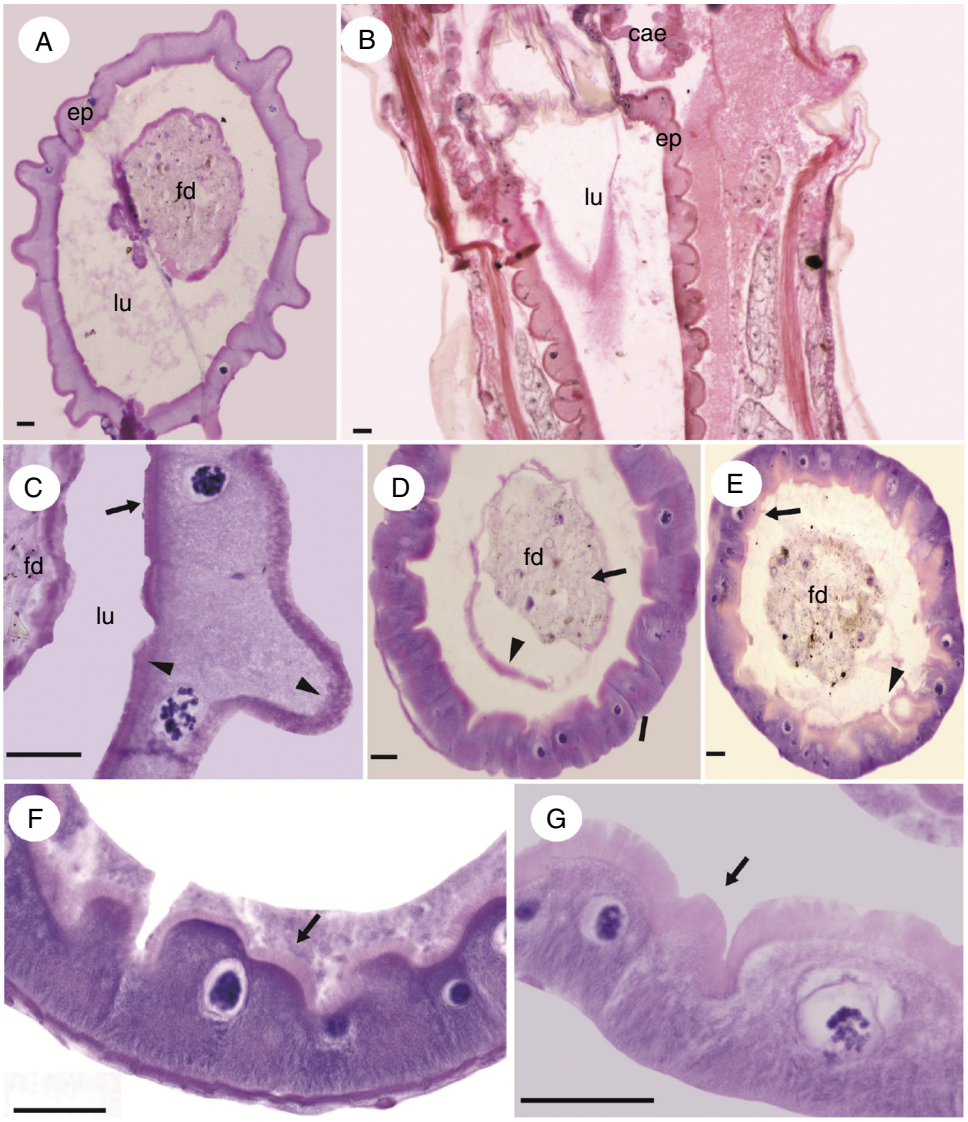

Fig. 3. Micrographs of the midgut of immature *Chironomus sancticaroli*. (A) Cross-section of the midgut region I; (B) longitudinal section of the midgut region I; (C) cells of the epithelium of the midgut region I, showing the little brush border area (arrow) and apical and basal eosinophilia of the cell (arrowhead); (D) cross-section of the midgut region II; (E) Cross-section of the region III of the midgut; (F) Brush border (arrow) and peritrophic matrix (arrowhead) in region II of the midgut and (G) Brush border (arrow) and cells in the process of secretion (arrowhead) in region III of midgut. cae: gastric caeca; ep: gut epithelia; fd: food; lu: lumen. Stain: Harris hematoxylin and eosin. Scale bar = 20 μm.

Following the Cuénot cells is the gastric caeca zone, formed by three or four crowns of fifteen diverticula, being the posterior diverticula significantly larger. They are formed by globose cells that are slightly smaller than the Cuénot cells, with granular eosinophilic cytoplasm, large spherical nuclei where the chromatin can be observed, and cells with extensive brush border (Fig. 2E).

In the transition between the foregut and the midgut is the estomodeal valve. This valve is characterized by a projection of the epithelium and muscle of the esophagus into the midgut, being composed by Cuénot cells and region of gastric caeca (Fig. 2F).

The midgut is divided into three regions with different cell types. Region I is formed by large cells with irregular shape. In cross-section, the epithelium has a star shaped contour, while the lumen has a circular shape (Fig. 3A). The basal region of the cell is rounded and convex, and protrudes between adjacent muscle fibers (Fig. 3B). In the apical and basal region of the cytoplasm of the cell is possible to see a more eosinophilic outline than the cytoplasm, which is granular. The brush border is not extensive (Fig. 3C). Region II has cubical cells with large nuclei and basophilic cytoplasm (Fig. 3D). Region III is formed by cubical cells, slightly smaller than the anterior region (Fig. 3E). The basal pole of region II cells displays heterogeneously stained regions in the form of striations (Fig. 3F). It is possible to observe a conspicuous brush border at the apical cell portion, more extensive than those of cells in the region I (Fig. 3F). The cytoplasm of these cells is strongly basophillic and has striations perpendicular to the base of the cell, being more evident than the ones on the anterior region (Fig. 3G). In the region below the nuclei and in the apical cell portion, the cytoplasm is granular and the brush border is conspicuous, representing a quarter of the size of the cells. Also, in region III, there are isolated regenerative cells located in the basal epithelium (Fig. 3G).

The first region of hindgut or valve region is formed by a cylindrical epithelium with basophilic cytoplasm, mid-basal nuclei and peripheral chromatin (Fig. 4A). The valve is formed by a cubical epithelium with eosinophilic cytoplasm and nuclei with not condensed chromatin (Fig. 4B and C). The region of the ileum is similar in structure to the esophagus, consisting of a thick circular muscle layer and a thin lining epithelium cells, formed by cells with imprecise boundaries, with large and spherical nucleus, and chromatin arranged in peripheral granules. The thin lining epithelium forms

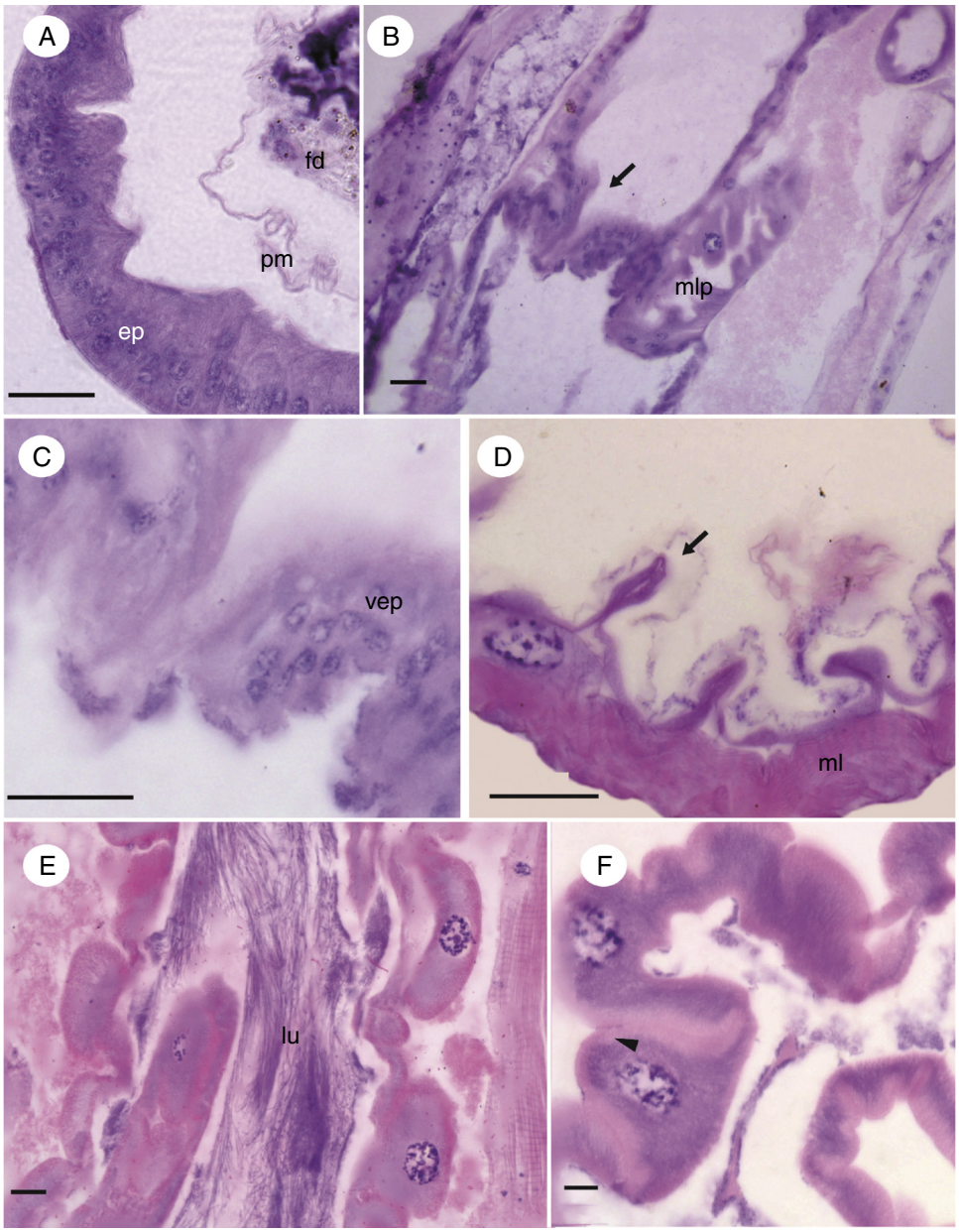

Fig. 4. Micrographs of hindgut immature of *Chironomus sancticaroli*. (A) cross-section between the transitional epithelium of the midgut and hindgut; (B) longitudinal section of the transition region between the mid and hindgut, showing the proctodeal valve (arrow); (C) in detail, epithelium of the proctodeal valve; (D) cross-section of ileum showing extensive muscle layer and the longitudinal folds formed by the epithelium (arrow); (E) longitudinal section of the colon and rectum; (F) cross-section of the epithelium of the colon and rectum demonstrating basal eosinophilia of the cell (arrowhead). p: epithelia; fd: food; lu: lumen; ml: muscle layer; mlp: Malpighian tubule, pm: perithrofic membrane; vep: valve epithelia. Stain: Harris hematoxylin and eosin. Scale bar = 20 μm.

numerous longitudinal folds lined by cuticle (Fig. 4D). In the final portion of the digestive tract, the colon and rectum can be observed. They have the same cellular morphology, composed of large cells with elongated basal region containing large spherical nucleus. The cytoplasm is slightly basophilic, with a eosinophilic basal and apical regions (Fig. 4E and F).

Salivary gland

The salivary glands are pairs, and slightly curved structures, following the dorsal wall of the body, located in the second and third larval thoracic segment (Fig. 1C). In addition, the glandular epithelial cells are arranged in a single layer, delimiting the large central lumen of the secretory portion, where is deposited the secretion that exhibits high affinity to eosin. These cells have large nuclei containing polytene chromosomes, the cytoplasm is uniform and strongly basophilic (Fig. 5A and B). The excretory duct opens into the esophagus, consisting of cubical epithelium and spherical nuclei with condensed chromatin and basophilic cytoplasm (Fig. 5C).

Excretory system

The species has four Malpighian tubules, with its proximal portion opening in the first region of the hindgut and a closed distal portion. These tubules are free in the body cavity, being bathed by hemolymph (Fig. 5D). The epithelium is composed of a single layer of pavement cells, with a large nucleus projected to the lumen of the tubule. The cytoplasm is granulose and eosinophilic and in the apical pole of the cell, a short brush border is easily observed (Fig. 5D and F).

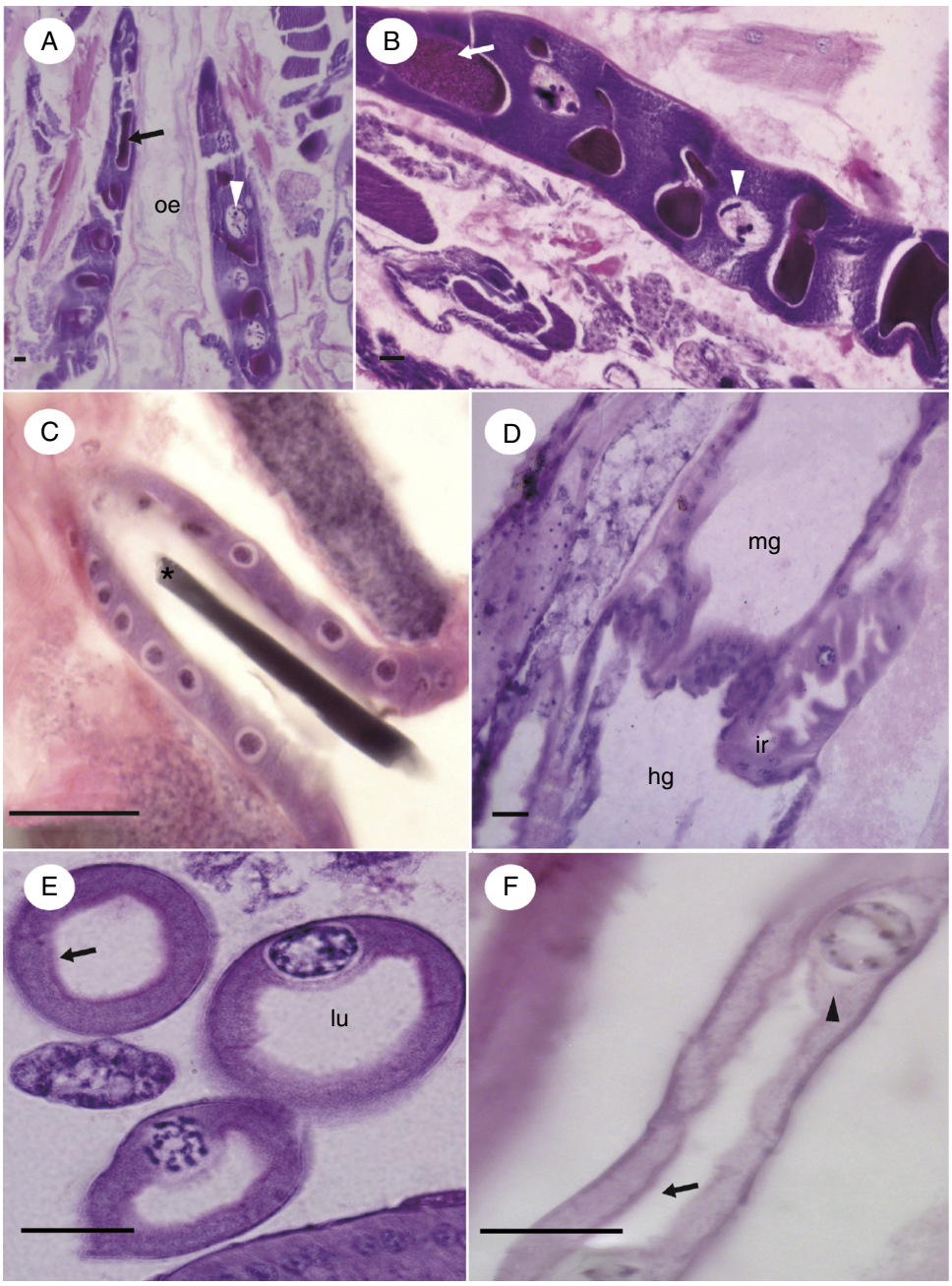

Fig. 5. Micrographs of structures attached to the digestive tract of immature *Chironomus sancticaroli.* (A, B) Tangential section of the salivary gland, showing parts of the lumen containing secretion (arrow) and cells, highlighting the nucleus with polytene chromosomes (arrowhead); (C) longitudinal section of salivary gland duct, note the presence of content in the lumen (*); (D) longitudinal section showing the insertion site of a Malpighian tubule in the digestive system; (E) cross-section of the tubules with the lumen and the brush border (arrow); (F) longitudinal section of a tubule with the brush border (arrow) and the projection of the nucleus into the lumen (arrowhead). hg: hindgut; ir: insertion region; lu: lumen; mg: midgut; oe: esophagus. Stain: Harris hematoxylin and eosin. Scale bar = 20 μm.

Nervous system

The central nervous system consists of brain, subesophageal ganglion and ventral nerve cord (with abdominal and thoracic ganglia). The brain is a bilobed organ located above the esophagus, being organized into cortical and medullar layer. The association of the neural lamella with the perineurium constitutes the nerve sheath (Fig. 6A). The ventral nervous cord is constituted by three thoracic ganglia and eight abdominal ganglia that are connected together and to the subesophageal ganglion by connective pairs, which are formed by the axons of the neurons enclosed by the neural lamella. The first abdominal ganglion is dislocated to the metathorax of the larvae, and the thoracic ganglia are slightly larger than the abdominals. The cellular structure of the ganglia in the nerve chain is similar to the one described for the brain, but the cell bodies, that are located in the ventral ganglion (Fig. 6B and C). The cortical region of brain shows the cell bodies of neurons, while the medullary region is characterized by the neuropile, a region formed by the axons of neurons (Fig. 6D). The frontal ganglion is located antertior to the brain and around the oral cavity (Fig. 6E).

Endocrine system or retrocerebral complex

The retrocerebral complex is composed mainly by the *corpora allata*, *corpora cardiaca*, and prothoracic gland (Fig. 1D). *Corpora allata* are paired spherical structures, consisting of glandular

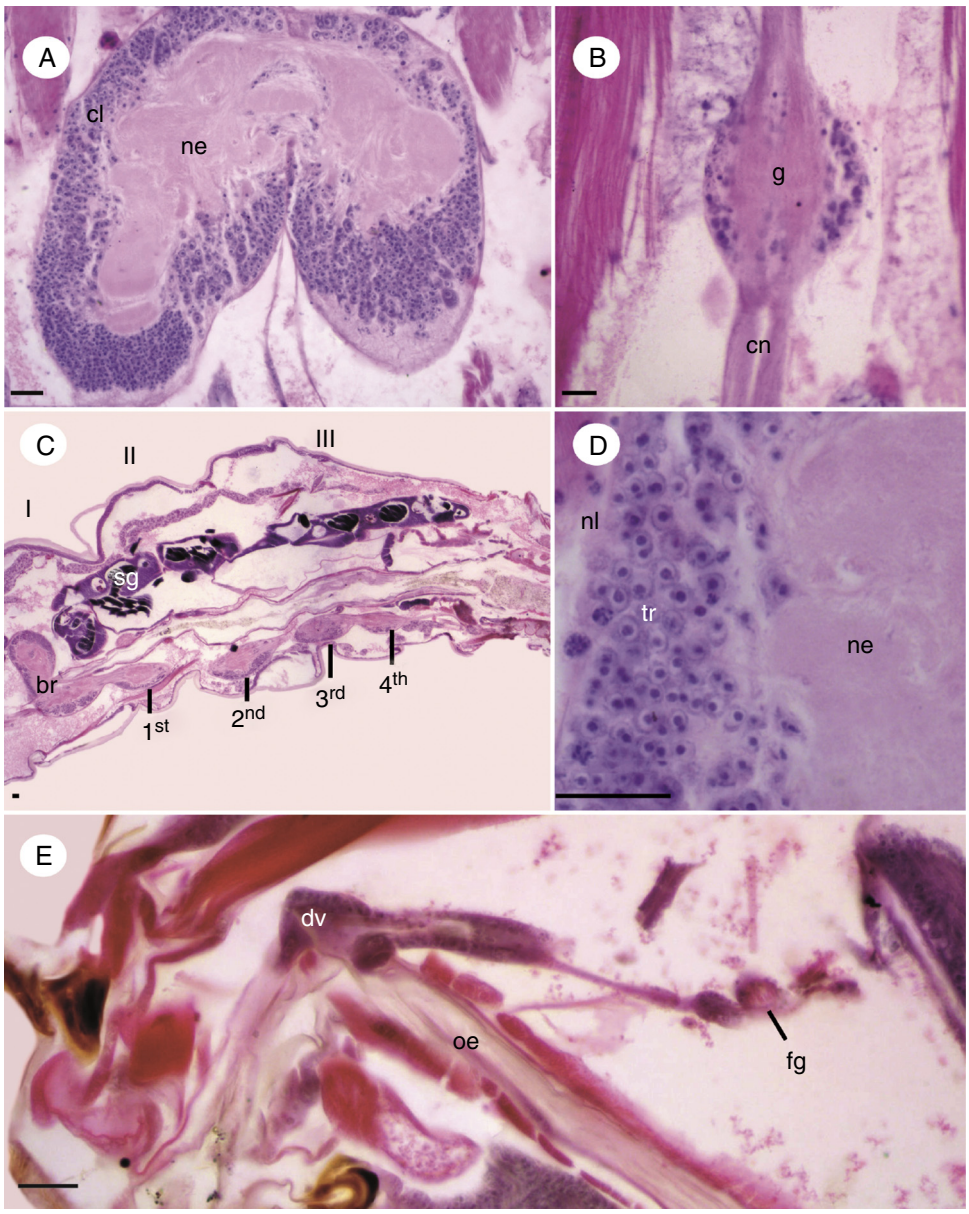

Fig. 6. Micrographs of nervous system structures of immature of *Chironomus sancticaroli*. (A) Longitudinal section showing the brain cortical and neuropile; (B) longitudinal section of a ganglion in the nerve cord and a connective sheaf formed by axons; (C) longitudinal section showing the brain, thoracic ganglia and the first abdominal ventral nerve cord (numbers); (D) cross-section of the brain; (E) longitudinal section of the cephalic region of the larva, in detail is the frontal ganglion, anterior to the brain. 1st: first thoracic gangliom, 2nd: second thoracic gangliom; 3rd: third thoracic gangliom, I–III: thoracic segments; 4th: first abdominal gangliom; br: brain; cl: cortical layer, cn: connective; dv: diverticulum; fg: frontal gangliom; g: gangliom, ne: neuropile; nl: neural lamella, oe: esophagus; sg: salivary gland, tr: trophoblastes. Stain: Harris hematoxylin and eosin. Scale bar = 20 μm.

epithelium formed by a few cells of varying sizes, with central nucleus and vacuolated cytoplasm. They are located close to the aorta, posterior to the brain and to the salivary gland (Fig. 7A). Prothoracic glands are formed by slightly pyramidal cells with basophilic cytoplasm and large nuclei, varying from spherical to oval, and with chromatin arranged in clumps. They are arranged longitudinally, involving the cephalic tracheae (Fig. 7B). The anterior postcerebral glands are pairs and unicellular structures. The larger cell has intensely stained nucleus, due to the condensation of chromatin, and cytoplasm with vacuoles and granules. They are located closely associated with the wall of the esophagus (Fig. 7C and D). *Corpora cardiaca* are paired structures, formed by a set of small irregularly shaped cells, with vacuolated cytoplasm and nuclei with condensed chromatin. They are located close to the cephalic dorsal trachea, between anterior post-cerebral glands and prothoracic gland (Fig. 7C and E).

Fat body

The fat body is constituted by oenocytes and trophocytes. In the parietal fat body, only in the abdominal segments, ventrally, occurs singly a modified trophocyte probably with hemoglobin contents (Fig. 8A). The oenocytes are located dispersed, occurring in gropus in the parietal fat body. These cells are large with globular aspect, bigger than trophocyte, but in smaller quantities (Fig. 8B). The hemoglobin cells and oenocytes have a stained similarly, the cytoplasm is acidophilus and the nucleus is central, but in the modified trophocytes have acidophilus cytoplasmics granules and the nucleus with chromatin clumps (Fig. 8C) while oenocytes have a perinuclear basophilic cytoplasm and in the nucleus, the chromatin is distributed in the periphery (Fig. 8D).

The parietal fat body is located between the epidermis and the intersegmental muscles, while the visceral fat body is distributed

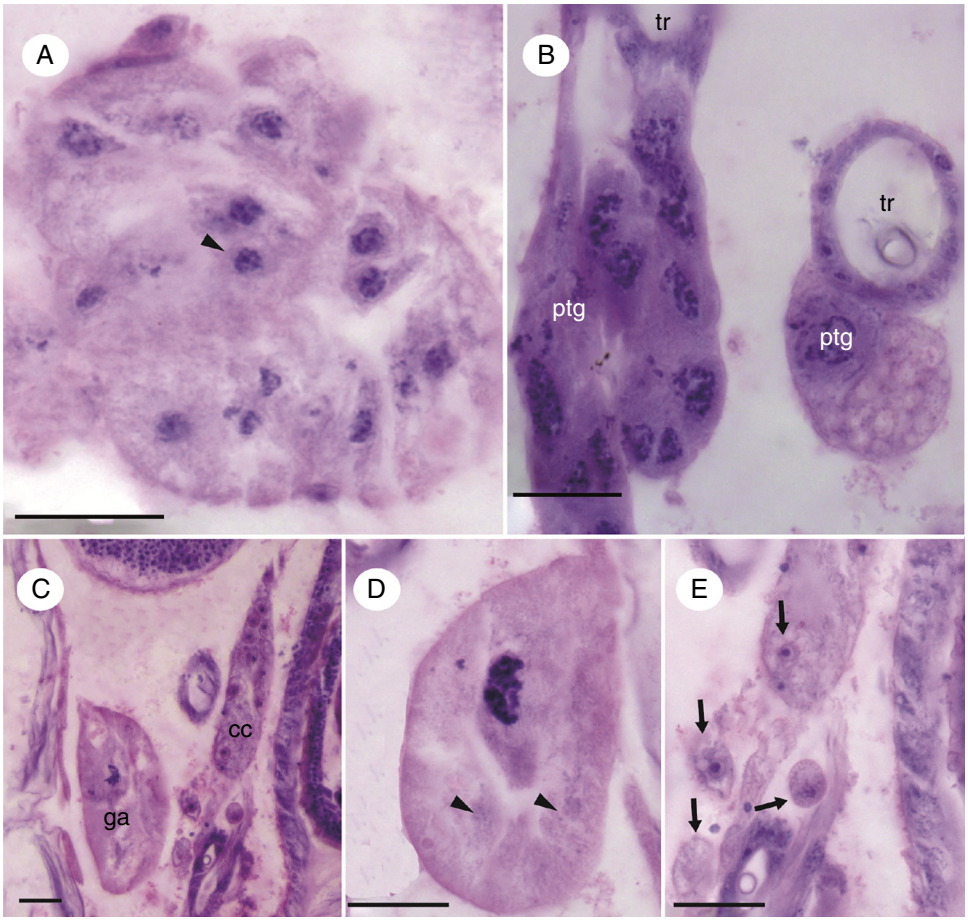

Fig. 7. Micrographs of glands from the retrocerebral complex of the immature *Chironomus sancticaroli*. (A) Longitudinal section of the corpora allata, showing the glandular epithelium, demonstrating the cell nucleus (arrowhead); (B) longitudinal section of the prothoracic gland; (C) longitudinal section showing the region of the complex, demonstrating the anterior postcerebral gland and the small group of cells that make up the corpora cardiac; (D) detail of the anterior postcerebral gland, note the granules in their cytoplasm (arrowhead); (E) detail of the small group of cells that make up the corpora cardiaca (arrows). cc: corpora cardiaca; ga: anterior postcerebral gland; ptg: prothoracic gland, tr: trachea. Stain: Harris hematoxylin and eosin. Scale bar = 20 μm.

between the internal organs of the larva. The parietal fat body has trophocytes and oenocytes, while the visceral is constituted only by trophocytes. The trophocytes of the parietal fat body are small cells and form a solid mass associated with the integument of the larva, while the visceral fat body cells are larger and form cellular masses that fill the spaces between the organs (Fig. 8E). The trophocytes present the cytoplasm with vacuolated appearance and no affinity for the hematoxylin and eosin stain, being stained by hematoxilyn in the perinuclear region. The nucleus is round and small, when compared to the cytoplasmic volume, and the chromatin is well condensed (Fig. 8F).

Circulatory system

The heart is located dorsally on the eighth abdominal segment, and it presents a chamber lined by a thin contractile muscle wall, which is difficult to observe in light microscopy. In the muscular wall, the ostia open up, which allows the entrance of the hemolymph (Fig. 9A). The aorta is located dorsally, above the digestive tract. It extends from the region of the heart to the head, with its cephalic end similar to a trumpet. The cells that form the wall of the aorta are pavement cells with little cytoplasm and flattened nucleus, containing condensed chromatin in its periphery (Fig. 9B). Throughout its length, it was observed valves that prevent backflow of hemolymph (Fig. 9C). The presence of pericardial cells associated with the aorta is also reported (Fig. 9D), as described in the excretory system. The pericardial cells are large, multinucleated, with eosinophilic cytoplasm and vacuoles, and found attached to the wall of the dorsal vessel (Fig. 9D).

Integument

The epidermis consists of a single layer of cubical cells that may form folds in the body of larvae, with eosinophilic cytoplasm and spherical nuclei with condensed chromatin. The cuticle lining the epidermis is made up of three layers, with the epicuticle being the outermost and the most difficult to see in light microscopy, the exocuticle the middle layer and the endocuticle the most intern one. The cephalic capsule is a strongly sclerotized structure, so it is possible to observe a thin endocuticle, a thicker layer of exocuticula and an extremely thin and difficult to observe epicuticle (Fig. 9E). In the body of the larvae, the cuticle is represented by a larger amount of endocuticle, in comparison with the thicknesses of layers of exocuticle and epicuticle (Fig. 9F).

Discussion

The majority of histological studies with Chironomidae histology were held at the end of the nineteenth century and the first half of the twentieth century. However, these authors only illustrated the general morphology of tissues, without detailing the structure of their cells (Miall and Hammond, 1900; Burtt, 1938;

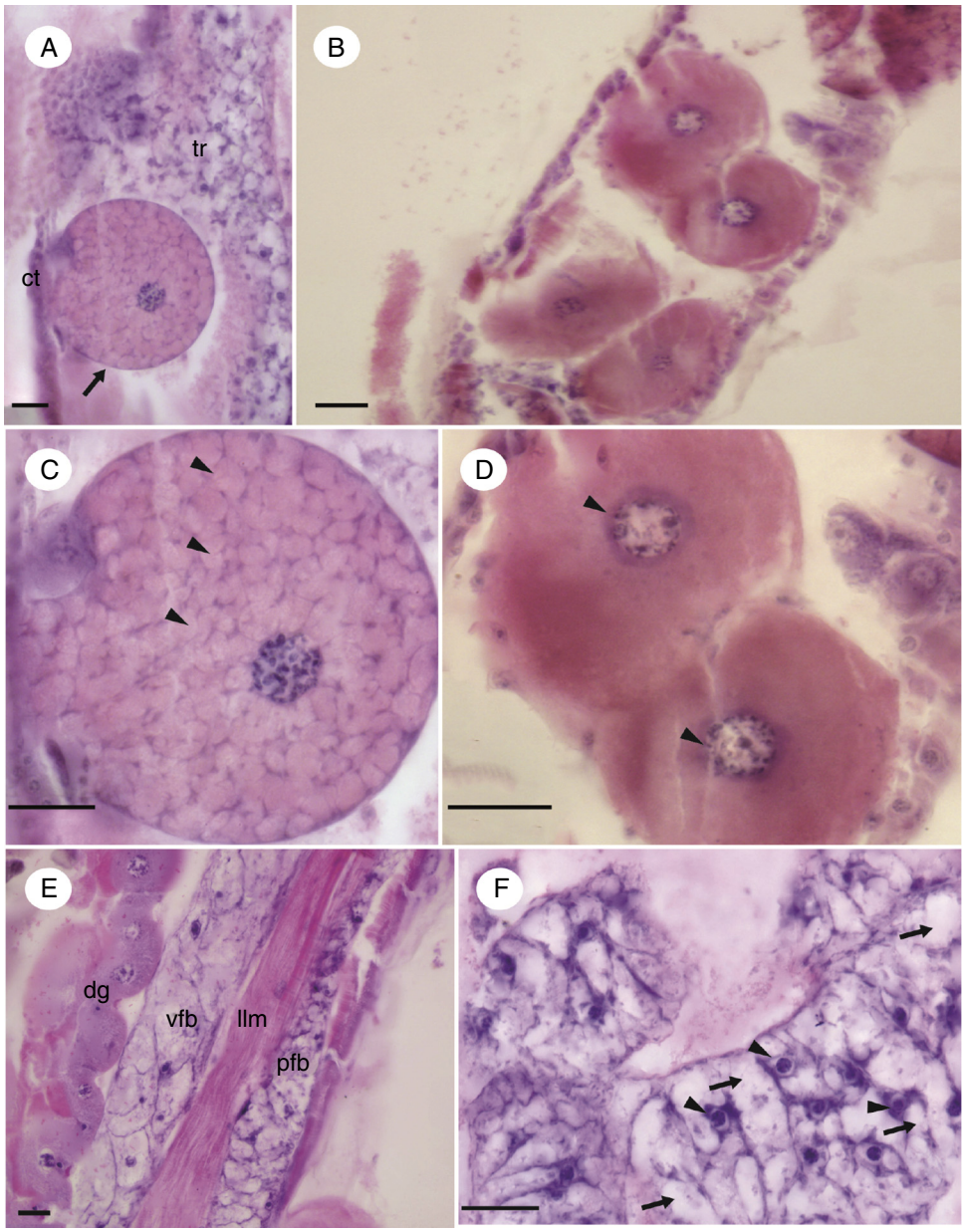

Fig. 8. Micrographs of the fat body of immature *Chironomus sancticaroli*. (A) Hemoglobin cell in parietal fat body; (B) oenocyte, arranged in groups distributed in the parietal fat body; (C) in detail, the hemoglobin cell presenting granules in its cytoplasm (arrowhead); (D) grouped oenocytes showing the basophilic perinuclear cytoplasm (arrowhead); (E) longitudinal showing the arrangement of the parietal and visceral fat body; (F) in detail the trophocytes, cells that are in greater quantity in this tissue, note the perinuclear cytoplasm (arrowhead) and the vacuoles filling the cell (arrows). ct: cuticle; dg: digestory tube; llm: longitudinal muscle layer; pfb: parietal faty body, tr: trophocytes; vfb: visceral body fat. Stain: Harris hematoxylin and eosin. Scale bar = 20 μm.

Possompès, 1948; Zee and Pai, 1944; Gouin, 1946; Possompès, 1946; Cazal, 1948; Pierson, 1956; Credland and Phillips, 1974; Credland and Scales, 1976; Credland, 1978; Panov, 1979; Seidman et al., 1986; Jarial, 1988; Nardi et al., 2009). Furthermore, the nervous, endocrine, circulatory systems and fat body were not detailed histologically for Chironomidae.

In ecotoxicological context, many xenobiotics can cause pathological changes in organs and systems of organisms. Histopathology is an important analytical tool between molecular, cellular and biochemical biomarkers and is considered an endpoint with relevant extrapolations on the population level. Histological approach is used mainly in organisms exposed to low concentrations, showing chronic effects. Such a damage assists in the elucidation of xenobiotics target organs (Chiang and Au, 2013). Histopathology is used as biomarker in studies involving several animal groups, predominantly fish (gill, liver, kidney, and gonads) in Aquatic Toxicology (Bernet et al., 1999). The advantage of working with this group is linked to the large amounts of tissue in each organ, which allows analysis of only one organ in the slide. But with some invertebrates, due to their body size, it is only possible to perform histological studies evaluating whole organism on the slide, requiring prior knowledge to identify target tissues.

The ecotoxicological studies with insects regarding Hymenoptera always refer to histological patterns under normal conditions of the model organism as related in previous studies (Cruz et al., 2010; Catae et al., 2014). But for Chironomidae larvae, these standards are not available. In studies with Hymenoptera, various tissues (midgut, Malpighian tubules, fat body, brain) were sensitive to various toxic substances, and in addition to these tissues other possible target systems have also

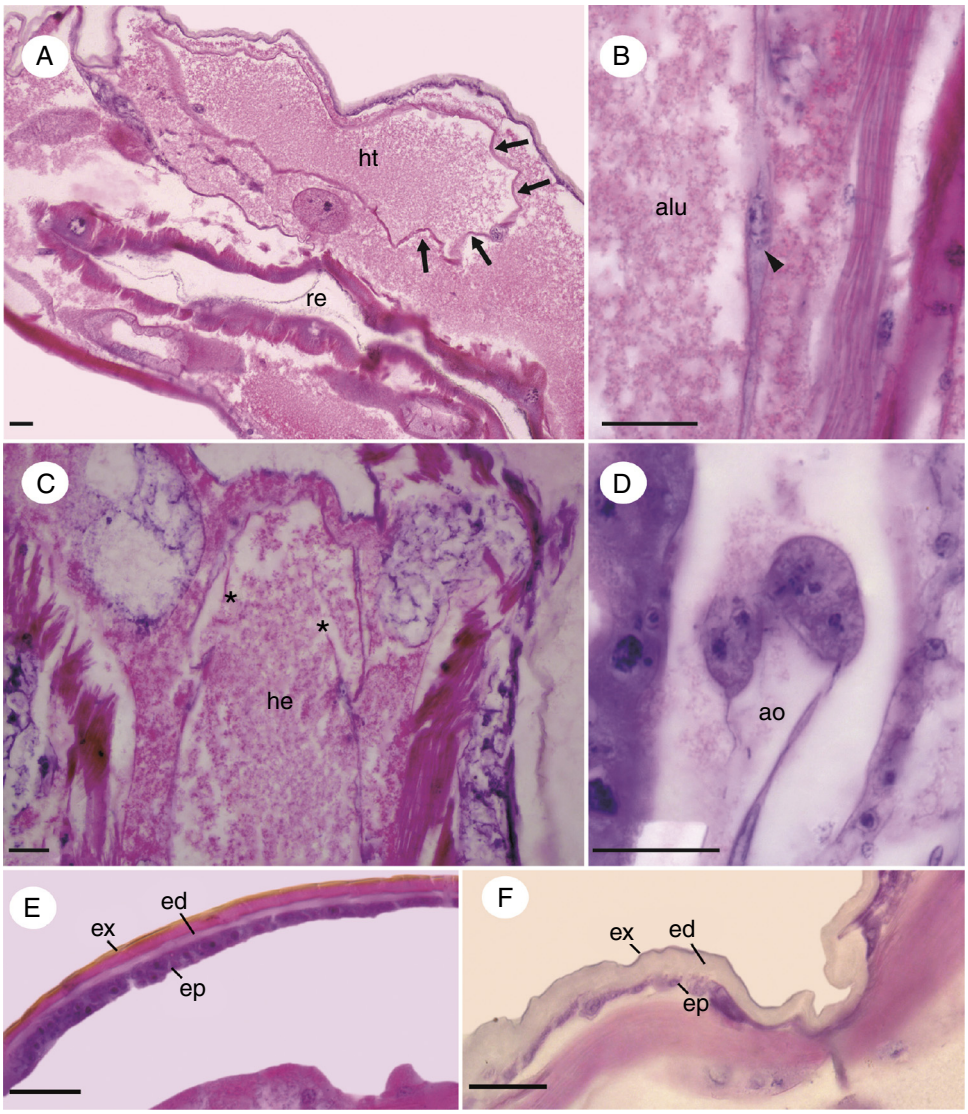

Fig. 9. Micrographs of the circulatory system and the integument of immature *Chironomus sancticaroli*. (A) Longitudinal section of the heart, note the ostium region (arrows); (B) longitudinal section of the region of the vessel wall with the cell of the vessel (arrowhead); (C) longitudinal section showing the aortic valves (*); (D) pericardial cells attaching to the wall of the aorta; (E) longitudinal section of the integument of the head capsule, showing the epithelium and the large amount of exocuticle; (F) longitudinal section of the integument of the body of the larva, note the greater amount of endocuticle. alu: aortic lumen; ao: aorta, ed: endocuticle; ep: epidermis, e.g. exocuticle; h: hemolymph, ht: heart; re: rectum. Stain: Harris hematoxylin and eosin. Scale bar = 20 μm.

been described in the present study (endocrine and circulatory system, salivary gland, integument) to allow easy identification of tissues and organs that may change in future histopathological analyzes of the Chironomidae larvae.

In a histopathological analysis, it is considered of great importance to study the types of changes that can occur in the cells of the model animal. The midgut appears as a target organ due to its role in absorption of orally acquired xenobiotic substances. In honeybee larvae (Cruz et al., 2010), it was observed vacuolation of the cytoplasm, dilation of the intercellular space, extrusion of cellular contents and chromatin compaction induced by exposure to boric acid and fipronil. In workers ants (Sumida et al., 2010), the same changes mentioned above were observed, in addition to narrowing the width of the epithelium, and nuclear pyknosis in the presence of boric acid.

The Malpighian tubules act in metabolite excretion, therefore also are sensitive to xenobiotics. Workers bees larvae and workers ants showed vacuolization of the basal cytoplasm of epithelial cells. Furthermore, chromatin condensation, nuclear pyknosis, irregular nuclei morphology, obstructed lumen and alteration of cell volume were observed in bee larvae (Ferreira et al., 2013; Rossi et al., 2013a; Sumida et al., 2010). Xenobiotics can change the energy metabolism of some organisms (Servia et al., 2006), and the fat body is responsible for this function, in addition to producing the enzymes that participate in the biotransformation of chemicals. That is why it may be a target organ for contaminants. In workers bees larvae, it was observed nuclear pyknosis in trophocytes exposed to boric acid and changes in cell volume in oenocytes exposed to paraquat (Cruz et al., 2010; Cousin et al., 2013).

Other tissues unrelated to absorption, metabolism and excretion of xenobiotics may also be targets for histological changes, such as the brain. Adult bees exposed to imidacloprid, there were alterations in pedunculated body cells and optic lobe, such as chromatin condensation, changes in cell volume and cell death (Rossi et al., 2013b).

Conclusions

This study presents a description of the histology of a biondicator of the quality of aquatic sediments in the absence of chemical

contaminants. We described peculiarities of some organs never detailed before to the group, which will be used as a reference for ecotoxicological studies; besides they assist in the understanding of the target organ of some xenobiotics.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, process 305038/2009-5) and Camila da Costa Senkiv for English review.

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